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Direct Association of p110β Phosphatidylinositol 3-Kinase with p85 Is Mediated by an N-Terminal Fragment of p110β

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Phosphatidylinositol (PI) 3-kinase is a heterodimeric enzyme of 85-kDa (p85) and 110-kDa (p110) subunits implicated in mitogenic signal transduction by virtue of its activation in cells transformed by diverse viral oncoproteins and treated with various growth factors. We have identified a domain in p110 that mediates association with p85 in vitro and in intact cells. A glutathione *S*-transferase fusion protein containing the N-terminal 171 amino acids of p110β bound to free p85 in cell lysates. This fusion protein also bound directly to p85 immobilized on nitrocellulose filters. An epitope-tagged fragment containing amino acids 31 to 150 of p110β associated with p85 upon expression in intact cells. Expression of either an N-terminal fragment of p110β or the p85 inter-SH2 domain, which mediates association with p110, reduced the association of endogenous PI 3-kinase activity with the activated platelet-derived growth factor receptor in intact cells. Hence, these defined regions of p85 and p110 mediate the interaction between the two subunits of PI 3-kinase.

Phosphatidylinositol (PI) 3-kinase is an enzyme which phosphorylates the 3′ OH of the inositol ring (51). PI 3-kinase associates with a variety of transforming viral oncoproteins and activated growth factor receptor tyrosine kinases (7, 8, 12–15, 20, 21, 26, 27, 35, 36, 38, 45, 46, 49, 50, 52). Transformed cells and cells treated with a variety of growth factors and cytokines exhibit elevated levels of 3′-phosphoinositides (2, 4, 6, 19, 28, 36, 39, 40, 48–50). Studies with mutant viral oncoproteins demonstrate that elevations in PI 3-kinase products are coincident with transformation competence (28, 40, 48, 49), suggesting that PI 3-kinase plays an integral role in mitogenic signaling. The products of PI 3-kinase are not substrates for phospholipase C (29, 41); it has been proposed that they may function directly as second messengers.

PI 3-kinase exists as a heterodimer of 85-kDa (p85) and 110-kDa (p110) subunits (3, 42). p85 contains two Src homology 2 (SH2) domains and one Src homology 3 (SH3) domain (9, 34, 43), which are domains that mediate interactions with tyrosine phosphoproteins and proline-rich regions, respectively (1, 31, 37). p85 has no intrinsic PI 3-kinase activity (34), and the SH2 domains of p85 bind to activated growth factor receptor tyrosine kinases and other tyrosine phosphoproteins (17, 23, 30, 32). Thus, p85 is thought to function as an adaptor protein that couples the catalytic p110 subunit to tyrosine phosphoproteins. Subsequent cDNA cloning has identified two distinct forms of p110 which have intrinsic PI 3-kinase activity and associate with p85 (16, 18).

The inter-SH2 domain of p85 mediates associations with p110 (18, 24). In this study, we identified an N-terminal domain in p110 which mediates direct interaction with p85 in vitro and in intact cells. Expression of either binding domain in intact cells reduced the association of endogenous PI 3-kinase activity with the activated platelet-derived growth factor (PDGF) receptor.

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MATERIALS AND METHODS

Construction of glutathione *S*-transferase (GST) fusion proteins. Appropriate fragments of a human p110β cDNA template (18) were amplified by using the PCR. Oligonucleotide primers were tailed with *Bam*HI and *Eco*RI restriction sites, except for those primers used to amplify regions containing internal *Bam*HI restriction sites. PCR products were cut with *Bam*HI and *Eco*RI or *Eco*RI alone and subcloned into pGEX2T (Pharmacia). Recombinant plasmids were screened with restriction enzymes and assayed for the ability to direct synthesis of appropriately sized GST fusion proteins. Fusion proteins were purified as previously described (44). Fusion proteins A to I (see Fig. 1) express the following regions of human p110β: A, amino acids 1 to 171; B, 171 to 312; C, 312 to 425; D, 422 to 740; E, 586 to 665; F, 586 to 882; G, 586 to 1070; H, 779 to 882; I, 779 to 1070.

Construction of expression vectors. An *Eco*RI-tailed oligonucleotide corresponding to the 5′ end of the hemagglutinin epitope in triplicate (47) and containing a methionine codon in a favorable Kozak consensus context (25) and *Hin*dIII-tailed oligonucleotides corresponding to regions surrounding amino acid 613 of human p85α (43) and amino acid 150 of human p110β (18) were used to amplify inserts from pINTTag and p110Tag, respectively (18). PCR products were cleaved with *Eco*RI and *Hin*dIII and subcloned into a cytomegalovirus enhancer-based expression vector (17) that had been cut with *Eco*RI and *Hin*dIII to generate p85INTTag and p110NTag. p85INTTag is identical to pINTTag (18), except that it encodes a protein lacking the 30 amino acids N terminal to the epitope tag which are encoded by pINTTag. p110NTag encodes the epitope tag fused to amino acids 31 to 150 of human p110β (18).

Expression vectors directing the synthesis of human p85 and the PDGF-β receptor have been described previously (17).

Cell culture and transfections. Human embryonic kidney fibroblasts (293 cells) were grown in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum in a 5% CO_2 incubator. Approximately 10^6 cells were plated on fibronectin-coated 10-cm-diameter dishes and grown overnight. Cells were transfected as previously described (5). Briefly, after preincubation in a 3% CO_2 incubator for 1 h, cells were transfected with 10 μg of plasmid DNA in 1 ml of 1× BES

[*N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid]-buffered saline (BBS; 2× BBS contains 50 mM BES [pH 6.95], 280 mM NaCl, and 1.5 mM Na_2HPO_4) and 0.125 M $CaCl_2$ per 10-cm dish. For coimmunoprecipitation assays, 5 μg of p110NTag and p85 cDNAs were transfected; for PI 3-kinase assays, 10 μg of p110NTag or p85INTTag cDNA and 1.25 μg of PDGF receptor cDNA were transfected. Transfected cells were incubated for 12 to 16 h in the presence of 3% CO_2, washed with phosphate-buffered saline (PBS), and incubated overnight in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum in the presence of 5% CO_2 prior to lysis. Cells which were to be treated with PDGF were starved overnight in Dulbecco's modified Eagle's medium containing 0.5% fetal bovine serum prior to treatment with 50 ng of PDGF per ml for 5 min at 37°C and subsequent lysis.

Antibodies. Rabbit polyclonal anti-p85 antibodies for blotting are directed against a GST fusion protein containing amino acids 265 to 523 of human p85α. Mouse monoclonal antibody 12CA5 (Babco) reactive to the influenza virus hemagglutinin epitope YPYDVPDYA (11) was used to detect epitope-tagged proteins. Tyrosine phosphoproteins were blotted and precipitated with rabbit polyclonal antiphosphotyrosine (anti-P-Tyr) antibodies. Rabbit polyclonal anti-GST antibodies were used to detect GST fusion proteins in direct protein blotting.

Cell lysis, immunoprecipitation, and immunoblotting. Cells were washed twice with ice-cold PBS prior to lysis with 400 μl of lysis buffer {50 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.5], 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], 10 μg of aprotinin per ml, 10 μg of leupeptin per ml, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 10 mM tetrasodium PP_i, 100 mM sodium fluoride} per 10-cm dish. Lysates were incubated for 5 min on ice and centrifuged for 15 min at 4°C and 16,000 × *g*. Supernatants were subjected to further analysis.

Equal amounts of total protein, as measured by the Bio-Rad protein assay, were precipitated with antibodies prebound to protein A-Sepharose beads (Zymed) in the presence of 20 mM HEPES (pH 7.5). Protein A-Sepharose-antibody complexes were incubated with cell lysates for 2 h at 4°C prior to immunoblot analysis or PI 3-kinase assay.

For immunoblot analysis, immunoprecipitates were washed three times with ice-cold lysis buffer, boiled in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer for 5 min, subjected to SDS–8% PAGE, transferred to nitrocellulose (MSI), and blotted as previously described (53). Antibodies were detected with ^{125}I-labeled protein A (ICN) or by enhanced chemiluminescence (New England Nuclear).

In vitro binding assays. Approximately 500 ng of fusion protein immobilized on glutathione-agarose beads (Sigma) was incubated with lysates corresponding to 100 μg of total protein from 293 cells transfected with a p85 cDNA expression vector (17). After 2 h at 4°C, the beads were washed three times with ice-cold lysis buffer and boiled for 5 min in the presence of SDS-PAGE sample buffer. Bound proteins were separated by SDS–8% PAGE, transferred to nitrocellulose, and immunoblotted with anti-p85 antibodies as described in the previous paragraph.

Direct protein blotting. Lysates from untransfected 293 cells or 293 cells transfected with p85 and epitope-tagged p110β cDNAs (5 μg of total protein) or 12CA5 precipitates from these lysates (100 μg of total protein in lysate subjected to immunoprecipitation) were processed for immunoblotting as already described, with minor modifications. Nitrocellulose filters were incubated with 1 μg of GST or fusion protein A (Fig. 1) per ml in Tris-buffered saline–bovine serum albumin (53) for 1 h at room temperature and washed once with Tris-buffered saline, twice with Tris-buffered saline–0.05% Triton X-100, and once with Tris-buffered saline (10 min each time) prior to incubation with anti-GST antibodies. The blot was processed to completion as described in the section on immunoblotting.

PI 3-kinase assay. Immunoprecipitates were assayed for PI 3-kinase activity as described previously (2). Briefly, immunoprecipitates were washed three times with PBS containing 1% Nonidet P-40, twice with 0.5 M LiCl–0.1 M Tris (pH 7.6), twice with TNE (10 mM Tris [pH 7.6], 100 mM NaCl, 1 mM EDTA), and twice with 20 mM HEPES (pH 7.5)–50 mM NaCl–5 mM EDTA–0.03% Nonidet P-40–30 mM tetrasodium PP_i–200 μM sodium orthovanadate–10 μg of aprotinin per ml–1 mM phenylmethylsulfonyl fluoride. A 50-μl volume of 1× kinase buffer (20 mM Tris [pH 7.6], 75 mM NaCl, 10 mM $MgCl_2$, PI [Avanti; 200 μg/ml] sonicated in 20 mM HEPES [pH 7.5], 20 μM ATP, 200 μM adenosine, 10 μCi of [γ-^{32}P]ATP [6,000 Ci/mmol; New England Nuclear] per sample) was added to each immunoprecipitate, and the samples were shaken at room temperature for 20 min. A 100-μl volume of 1 N HCl was added to stop the reaction. Lipids were extracted with 200 μl of 1:1 (vol/vol) chloroform-methanol. The organic phase was dried, resuspended in 10 μl of 1:1 (vol/vol) chloroform-methanol containing a PI 4-phosphate standard, and spotted on a silica gel 60 thin-layer chromatography plate (Merck). Chromatography was performed in chloroform–methanol–4.0 M NH_4OH (9:7:2 [vol/vol/vol]), and labeled PI 3-phosphate was visualized by autoradiography.

RESULTS

The N-terminal 171 amino acids of p110β mediate direct association with p85 in vitro. We have previously identified the inter-SH2 region of p85 as a binding site for p110β (18). To identify domains of p110β involved in p85 binding, we constructed GST fusion proteins expressing various fragments of p110β. The different constructs are depicted schematically in Fig. 1. Fusion proteins were expressed in *Escherichia coli* and purified by glutathione-agarose affinity chromatography. Immobilized fusion proteins were incubated with lysates from 293 cells overexpressing human p85α. Bound proteins were separated by SDS-PAGE, transferred to nitrocellulose, and blotted with anti-p85 antibodies.

Purified fusion proteins were visualized by SDS-PAGE followed by Coomassie blue staining (Fig. 2A); the same amount of protein was used in the binding assay. p85 was appropriately overexpressed in lysates of 293 cells (Fig. 2B, lanes 1 and 2). Construct A, which contains the N-terminal 171 amino acids of p110β, bound to p85, whereas fusion proteins containing fragments spanning the rest of p110β did not bind detectably to p85 (lanes 3 to 11). GST itself also did not bind to p85 in this assay (data not shown).

Native PI 3-kinase migrates as a complex of approximately 200 kDa on gel filtration chromatography (3), suggesting that the association between p85 and p110 is direct and not mediated by other proteins. To determine whether this association is indeed direct, we assayed construct A (Fig. 1) for the ability to bind to p85 directly on a nitrocellulose filter. Lysates and anti-Tag precipitates of lysates from 293 cells coexpressing p85 and epitope-tagged p110β were subjected to direct protein blotting with either fusion protein A or GST (Fig. 3). Blotting of lysates and anti-Tag precipitates from untransfected and

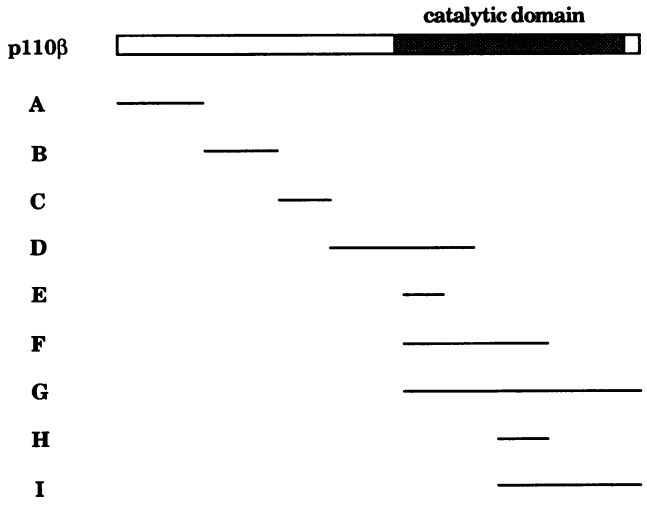

FIG. 1. Schematic diagram of GST-p110β fusion proteins. The shaded region representing the putative catalytic domain of p110β is based on amino acid sequence comparison among the three known PI 3-kinases (18). The GST portion of each fusion protein is not shown.

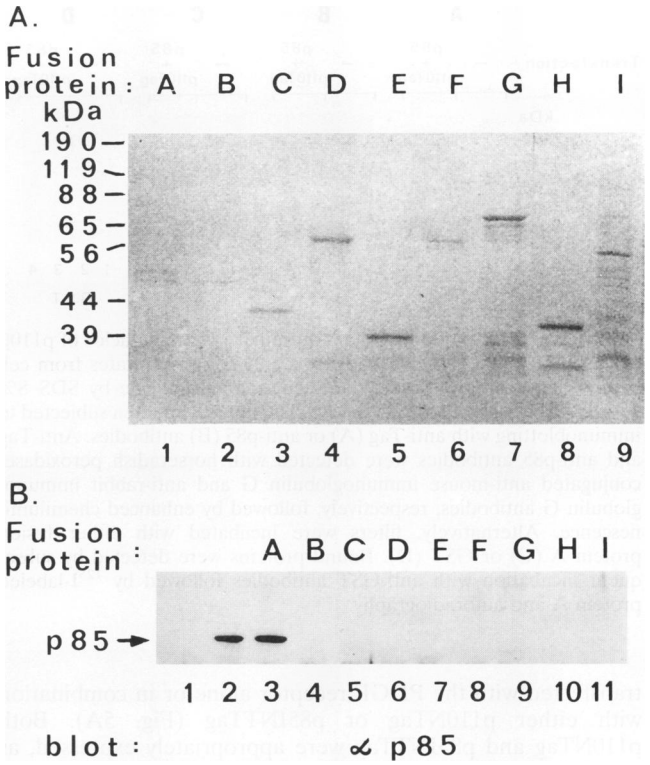

FIG. 2. (A) Coomassie blue-stained GST-p110β fusion proteins analyzed by SDS–10% PAGE. Fusion proteins are designated as in Fig. 1. Approximately 0.5 μg of protein was analyzed in each lane. The same amount of fusion protein was used for in vitro binding studies. (B) Binding of p85 to GST-p110β fusion proteins. Lysates from 293 cells transfected with a human p85 cDNA (43) were incubated with immobilized GST-p110β fusion proteins. Bound proteins were washed, separated by SDS–8% PAGE, and blotted with anti-p85 antibodies. Antibodies were detected with anti-rabbit immunoglobulin G antibodies conjugated to horseradish peroxidase followed by enhanced chemiluminescence. Five-microgram samples of lysate protein from untransfected and p85-transfected cells were analyzed in lanes 1 and 2, respectively.

transfected 293 cells with anti-Tag (Fig. 3A) and anti-p85 (Fig. 3B) antibodies demonstrated appropriate overexpression of epitope-tagged p110β (Fig. 3A) and p85 (Fig. 3B). p110β and p85 migrate slightly more slowly than the 119- and 88-kDa markers, respectively, when analyzed by SDS–8% PAGE (17, 18).

We have previously shown that p85 and epitope-tagged p110β are able to associate in intact cells (18). Direct protein blotting of the same samples with construct A followed by incubation with anti-GST antibodies and ^{125}I-labeled protein A demonstrated that construct A associated specifically with a protein that comigrates with p110β-associated p85 and was detectable only in lysates and anti-Tag precipitates from 293 cells overexpressing p85 and epitope-tagged p110β (Fig. 3C). GST itself did not bind directly to any proteins in this assay (Fig. 3D).

Amino acids 31 to 150 of p110β are sufficient for association with p85 in intact cells. Comparison of the N-terminal regions of p110β (18) and bovine p110 (16) reveals that the region comprising amino acids 32 to 150 of p110β is 50% identical to the analogous region of bovine p110. Taken together with the observation that the p85 inter-SH2 domain mediates interactions with both p110β (18) and the murine homolog of bovine p110 (24), this suggests that amino acids 32 to 150 of p110β should be sufficient to mediate association with p85. Thus, this region of p110β was amplified from p110Tag (18) and subcloned into an expression vector to generate p110NTag. 293 cells cotransfected with p110NTag and p85 cDNAs were lysed, and the lysates were assayed for coprecipitation of p85 with the epitope-tagged N-terminal fragment of p110β (Fig. 4).

p85 and p110NTag were appropriately overexpressed, as demonstrated by anti-p85 blotting of anti-p85 precipitates and anti-Tag blotting of anti-Tag precipitates (Fig. 4, lanes 2, 4, 7, and 8). The protein encoded by p110NTag migrated with mobility on SDS-PAGE consistent with its predicted molecular mass of approximately 18 kDa (Fig. 4, lanes 7 and 8).

No proteins recognized by the anti-Tag antibody were present in anti-p85 precipitates of lysates from untransfected cells or from cells transfected with either p85 or p110NTag alone (Fig. 4, lanes 1 to 3). However, upon coexpression of p85 and p110NTag, anti-Tag blotting revealed the presence of a protein in anti-p85 precipitates which comigrated with p110NTag (Fig. 4, compare lane 4 with lanes 7 and 8).

Likewise, p85 was not detectable in anti-Tag precipitates of lysates from untransfected cells or from cells overexpressing p85 alone (Fig. 4, lanes 5 and 6). Expression of p110NTag alone resulted in coprecipitation of endogenous p85 with p110NTag (Fig. 4, lane 7). Again, coexpression of p85 and p110Tag resulted in the coprecipitation of p85 with p110Tag (Fig. 4, lane 8).

The p85 inter-SH2 domain and an N-terminal fragment of p110β reduce association of endogenous PI 3-kinase activity with the activated PDGF receptor. Overexpression of p85 abrogates association of endogenous PI 3-kinase with activated PDGF receptors (17). To determine whether overexpression of minimal binding domains of p85 and p110 would achieve the same effect, either p85INTTag or p110NTag was coexpressed with the PDGF receptor in 293 cells. Lysates from PDGF-treated cells were precipitated with anti-P-Tyr antibodies, and immunoprecipitates were subjected to PI 3-kinase assay (Fig. 5).

Anti-P-Tyr blotting of cell lysates indicated that PDGF receptor tyrosine autophosphorylation was comparable in cells

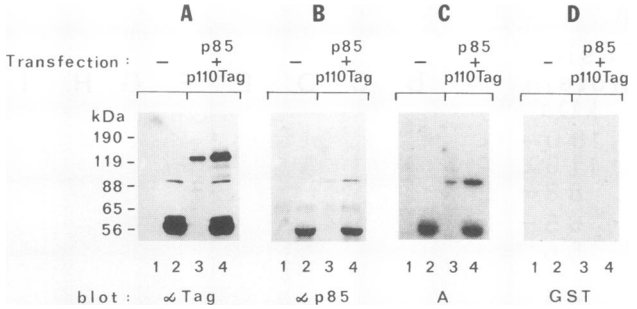

FIG. 3. Direct binding of the N-terminal 171 amino acids of p110β to p85. Cell lysate (lanes 1 and 3) or anti-Tag precipitates from cell lysates (lanes 2 and 4) were analyzed in quadruplicate by SDS–8% PAGE and transfer to nitrocellulose. The filters were then subjected to immunoblotting with anti-Tag (A) or anti-p85 (B) antibodies. Anti-Tag and anti-p85 antibodies were detected with horseradish peroxidase-conjugated anti-mouse immunoglobulin G and anti-rabbit immunoglobulin G antibodies, respectively, followed by enhanced chemiluminescence. Alternatively, filters were incubated with either fusion protein A (C) or GST (D). Bound proteins were detected by subsequent incubation with anti-GST antibodies followed by ^{125}I-labeled protein A and autoradiography.

transfected with the PDGF receptor alone or in combination with either p110NTag or p85INTTag (Fig. 5A). Both p110NTag and p85INTTag were appropriately expressed, as determined by anti-Tag blotting of cell lysates (data not shown). As previously demonstrated (17), assays of PI 3-kinase activity in anti-P-Tyr precipitates from cell lysates demonstrated that treatment of PDGF receptor-transfected 293 cells with PDGF resulted in recruitment of PI 3-kinase activity into the anti-P-Tyr-precipitable fraction (Fig. 5B, lanes 1 and 2). Coexpression of either p110NTag or p85INTTag reduced the amount of PI 3-kinase recruited into the anti-P-Tyr-precipitable fraction in response to PDGF treatment (Fig. 5B, lanes 3 to 6).

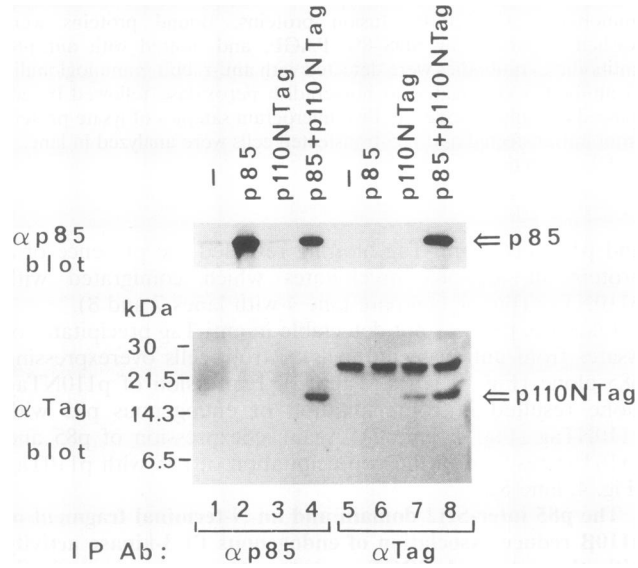

FIG. 4. Association of a protein fragment comprising amino acids 31 to 150 of p110β with p85 in intact cells. 293 cells were transfected, and 500 μg of cell lysate protein was precipitated with either anti-p85 (lanes 1 to 4) or anti-Tag (lanes 5 to 8) antibodies. Immunoprecipitates were split into equal portions, subjected to SDS–8% PAGE (top panel) or SDS–15% PAGE (bottom panel), and immunoblotted with anti-p85 (top panel) or anti-Tag (bottom panel) antibodies. Anti-p85 and anti-Tag antibodies were detected with horseradish peroxidase-conjugated *Staphylococcus aureus* protein A and horseradish peroxidase-conjugated anti-mouse immunoglobulin G antibodies, respectively, followed by enhanced chemiluminescence.

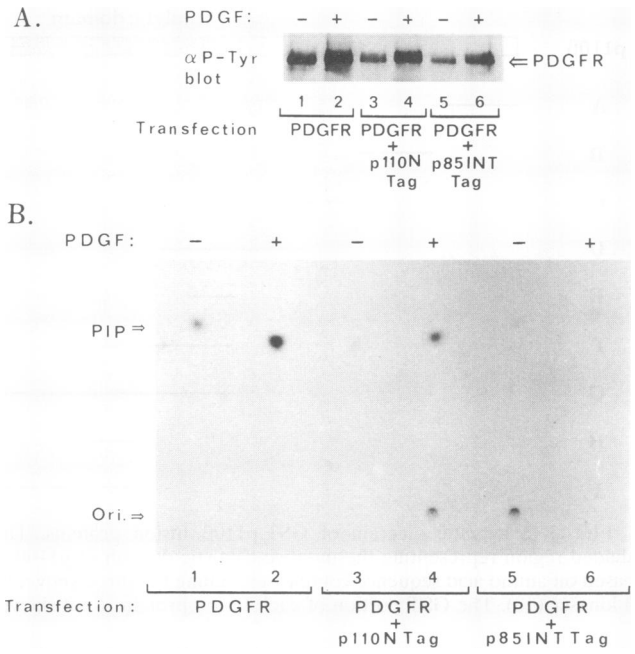

FIG. 5. Inhibition of PDGF-dependent PI 3-kinase recruitment to the P-Tyr fraction by expression of an N-terminal fragment of p110β or the p85 inter-SH2 domain. (A) PDGF-dependent receptor tyrosine autophosphorylation. 293 cells transfected with various constructs were treated with PDGF, and 8 μg of total lysate protein was analyzed by SDS–8% PAGE followed by blotting with anti-P-Tyr antibodies. Antibodies were detected with horseradish peroxidase-conjugated *S. aureus* protein A followed by enhanced chemiluminescence. (B) PDGF-dependent recruitment of PI 3-kinase activity to the P-Tyr fraction. A 200-μg sample of lysate from 293 cells transfected with various constructs and treated with PDGF was precipitated with anti-P-Tyr antibodies, and immunoprecipitates were assayed for PI 3-kinase activity. PDGFR, PDGF receptor; PIP, PI phosphate standard; Ori., origin.

DISCUSSION

We have identified the N-terminal region of p110β as a binding domain for p85. The N-terminal 171 amino acids of p110β (Fig. 1) were sufficient to mediate direct interactions with p85 (Fig. 2 and 3). A fragment containing amino acids 31 to 150 of p110β mediated association with p85 in intact cells (Fig. 4). Both this fragment and the inter-SH2 domain of p85 reduced association of endogenous PI 3-kinase activity with activated PDGF receptors when expressed in 293 cells (Fig. 5).

Although our data indicate that the N terminus of p110β contains a p85-binding domain, we have not identified a minimal binding domain. Fusion proteins expressing approximately 40-amino-acid fragments of the region between amino acid residues 31 to 150 in p110β did not bind to p85 in vitro (data not shown). In addition, we cannot exclude the possibility that another domain in p110β contributes to its association with p85.

The direct binding of p85 and p110β was confirmed by direct protein blotting with construct A (Fig. 3). Four observations support the specificity of this direct interaction. Construct A bound directly to p85 (Fig. 3C), whereas GST did not (Fig. 3D). Although both p85 and epitope-tagged p110β were overexpressed (Fig. 3A and B, lanes 3 and 4), construct A bound only to p85 (Fig. 3C). Also, the amount of construct A bound to p85 is proportional to the quantity of p85 present in lysates and anti-Tag precipitates from lysates of cells overexpressing p85 and epitope-tagged p110β (compare lanes 3 and 4 in Fig. 3C with the same lanes in Fig. 3B). Finally, this binding was strictly dependent on the overexpression of p85 (Fig. 3C, compare lanes 1 and 2 with lanes 3 and 4) and was enhanced by precipitation with anti-Tag antibodies (Fig. 3C, compare lanes 3 and 4). Our inability to detect binding of construct A to endogenous p85 (Fig. 3C, lane 1) is likely a consequence of the small amounts of total lysate protein analyzed; this quantity of endogenous p85 was also not detectable by anti-p85 blotting (Fig. 2B, lane 1, and 3B, lane 1). We also demonstrated that construct A binds directly to p85INTTag in vitro (data not shown), indicating that small domains in p85 and p110β suffice to mediate heterodimer formation.

Binding of GST–inter-SH2 to p110β was not detected in a direct protein blotting assay (data not shown), despite the ability of this fusion protein to bind to epitope-tagged p110β in lysates of 293 cells (18). This suggests that denaturation of p110β by SDS-PAGE abolishes elements of the structure of the native protein which are required for association with p85.

The association of the N-terminal domain of p110β with p85 was confirmed in intact cells by transient expression of this domain in 293 cells (Fig. 4). Although endogenous p85 was detected in anti-Tag precipitates of lysates from cells expressing p110NTag alone, p110NTag was not detectable in anti-p85 precipitates from the same cell lysates (Fig. 4, compare lanes 7 and 3). This is likely a consequence of the fact that much of the p85 precipitated is associated with endogenous p110 species; the relative amount of p110NTag associated with p85 depends on the efficiency of transfection and the level of p110NTag expression relative to levels of endogenous p110.

Although expression of p110NTag or p85INTTag reproducibly inhibited association of PI 3-kinase activity with activated PDGF receptors, it did not abolish this association completely (Fig. 5 and data not shown). Further studies are necessary to elucidate the nature of interactions between different isoforms of p85 and p110.

These results, in combination with those of previous studies on PI 3-kinase, can be incorporated into a model of interactions that govern PI 3-kinase association with activated PDGF receptors (Fig. 6). On the basis of this model, overexpression of either the N-terminal domain of p110 or the p85 inter-SH2 domain inhibits the association of endogenous PI 3-kinase with the activated PDGF receptor by uncoupling endogenous, full-length p110 from p85.

Interestingly, although bovine p110 has PI 3-kinase activity when expressed alone in insect cells, it is active as a PI 3-kinase only when coexpressed with p85 in COS-1 cells (16). Thus, coexpression of p85 functions either to counteract inhibition of bovine p110 or to activate p110. Since coexpression of p85 and bovine p110 results in the formation of p85-p110 complexes (16), the most parsimonious explanation for the effect of p85 on p110 PI 3-kinase activity is that p85 binding per se may activate the intrinsic PI 3-kinase of bovine p110. However, this has not been tested directly.

Epitope-tagged p110β is active as a PI 3-kinase both in the absence and in the presence of coexpressed p85 (18). Although

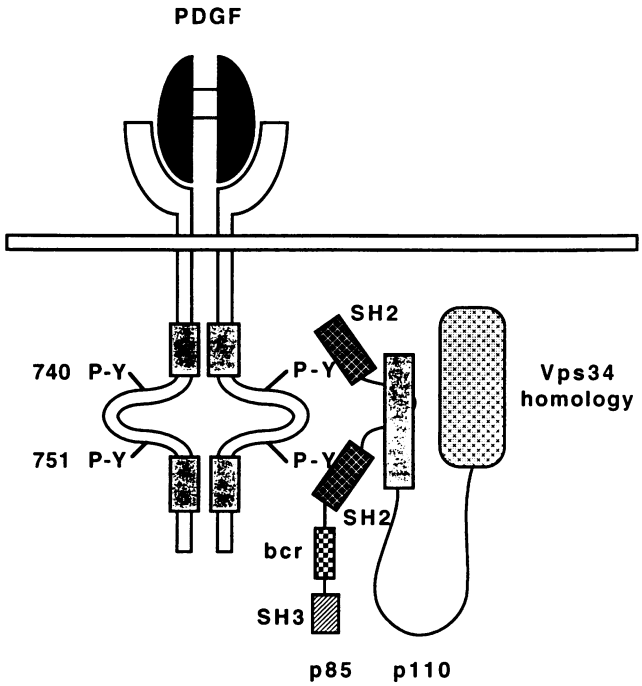

FIG. 6. Model of PI 3-kinase association with activated PDGF receptors. The SH2 domains of p85 are shown interacting with the PI 3-kinase-binding sites in the kinase insert of the activated receptor (10, 17, 22, 23, 30), and the p85 inter-SH2 domain is shown associating with the N-terminal region of p110. The SH3 and Bcr homology domains in p85 (9, 34, 43) and the region of p110 homology to Vps34 (16, 18) are indicated.

coexpression of p85 and p110β resulted in increased steady-state levels of p110β, the specific PI 3-kinase activity of p110β was unchanged (18). In addition, expression of p110β alone resulted in an increase in the PI 3-kinase activity present in anti-p110β precipitates, and coexpression of the p85 inter-SH2 domain had no effect on this activity (18). Thus, at this level of analysis, it appears that p110β is an active PI 3-kinase at very low stoichiometry of, or perhaps independently of, p85. In contrast, bovine p110 seems to require the presence of stoichiometric amounts of p85 for PI 3-kinase activity (16). These observations suggest that although p85 associates with both the murine homolog of bovine p110 and p110β through the p85 inter-SH2 domain (18, 24), the regulation of distinct p110 isoforms may differ. Confirmation of these preliminary observations requires activity assays of purified bovine p110 and p110β expressed in heterologous systems (such as in insect cells) in the presence and absence of coexpressed p85.

The role of PI 3-kinase in mammalian cells is not well understood. Studies of growth factor receptor point mutants which do not bind PI 3-kinase are complicated by the observation that different signaling molecules may share receptor-binding sites (33). The ability of both the p85 inter-SH2 domain and the p110β N-terminal domain to reduce the association of endogenous PI 3-kinase with activated PDGF receptors suggests that they may be used as dominant negative mutants to inhibit PI 3-kinase-mediated signaling specifically. Our efforts to overexpress full-length p85 or the p85 inter-SH2 domain stably in mammalian cells were unsuccessful (data not shown), suggesting that uncoupling of p85 and p110 is toxic to cells. Nonetheless, the identification of minimal domains in p85 and p110 involved in PI 3-kinase interactions should prove

useful in further studies on the roles and regulation of PI 3-kinase in mammalian cell physiology.

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